IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Yasuhiro NISHITANI et al.
Title:	CEPHEM COMPOUNDS HAVING BROAD ANTIBACTERIAL SPECTRUM
Appl. No.:	10/507,502
International Filing Date:	9/13/2004
Examiner:	Mark L. Berch
Art Unit:	1624
Confirmation Number:	6365

DECLARATION UNDER 37 C.F.R. § 1.132 OF YOSHINORI YAMANO

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, YOSHINORI YAMANO, do hereby make the following Declaration:

1. I received a PhD degree in the Graduate School of Agriculture, Kyoto University in March, 1994.

2. I have been employed by Shionogi & Co., Ltd., since 1986. I was working at the Research Division of Research Laboratories, Shionogi & Co., Ltd., since April 1986. From April, 2005, I am a head of Infectious Diseases Department.

3. I have extensive experience in the field of infectious diseases treatment as evidenced by my education and my 21 years of professional experience at the Research Division of Research Laboratories, Shionogi & Co., Ltd.

4. I am an inventor of the invention disclosed in US patent application no. 10/507,502. My employer, Shionogi & Co., Ltd. owns the patent application no. 10/507,502. Other than my regular salary, I have not been provided additional compensation for preparing this declaration.

5. I am able to read and understand the English language when it is written.

6. I have read the Office Action dated March 22, 2007, in the US application no. 10/507,502.

7. I have read the references US patents Nos. 4,788,185, 4,427,677, 4,559,102, Kawabata (WO97/41128) and Nishimura (Journal of Antibiotics, Vol. 45, 1992, p. 485-499) cited in the Office Action dated March 22, 2007, in the US application no. 10/507,502.

8. Under my supervision and control, the following experiments were conducted:

Nine series of compounds were tested for inhibitory activity against a broad spectrum of bacterial strains. Each of the nine series included three compounds labeled A, B and C. Within each of the nine series; the compound labeled A is a compound of formula (I) as defined in claim 1 of US no. 10/507,502, with T=S, X=Cl, R^2 = methyl, R^1 = hydrogen; the compound labeled B has R^2 = hydrogen, but otherwise is identical to the corresponding compound labeled A; the compound labeled C has R^1 = methyl, but otherwise is identical to the corresponding compound labeled A. Z^+ group as defined in claim 1 of US application no. 10/507,502 is the same for compounds A, B, and C within a single series, but is different for compounds belonging to different series.

Inhibitory activity of each of the compounds A, B and C in each of the nine series was determined against the following fifteen bacterial strains:

1) *Staphylococcus aureus* Smith (S. aureus SMITH);

2) *Staphylococcus aureus* SR3637(H-MRSA) (S. aureus SR3637(H-MRSA));

3) *Staphylococcus aureus* SR20067(H-MRSA) (S. aureus SR20067(H-MRSA));

4) *Streptococcus pneumoniae* SR16675(PRSP) (S. pneumoniae SR16675(PRSP));

5) *Escherichia coli* NIHJ JC-2 (E. coli NIHJ JC-2);

6) *Escherichia coli* SR21003(Toho2) (E. coli SR21003(Toho2));

7) *Enterobacter cloacae* ATCC 13047 (E. cloacae ATCC 13047);

8) *Enterobacter cloacae* SR4321(Bla++) (E. cloacae SR4321(Bla++));

9) *Pseudomonas aeruginosa* SR24 (P. aeruginosa SR24);

10) *Pseudomonas aeruginosa* SR24-12(bla+) (P. aeruginosa SR24-12(bla+));

11) *Pseudomonas aeruginosa* SR5393 (P. aeruginosa SR5393);

12) *Pseudomonas aeruginosa* SR6554 (IPM-R) (P. aeruginosa SR6554 (IPM-R));;

13) *Haemophilus influenzae* ATCC49766 (H. influenzae ATCC49766);

14) *Haemophilus influenzae* SR11435 (BLNA1) (H. influenzae SR11435 (BLNA1));

15) *Branhamella catarrhalis* ATCC43617(Bla +) (B. catarrhalis ATCC43617(Bla +)).

EXPERIMENTAL DETAILS

The antibacterial activities of the compounds were determined by the agar dilution method described below.

Preparation of serial two-fold dilutions of compound solutions

Each of the compounds was dissolved in distilled water (DW) and then the solution were further diluted with DW to make serial 2-fold dilutions at concentrations of 1280 - 0.04 μg/mL.

Preparation of inoculum

Bacterial colonies of each of the fifteen strains were grown at 37°C in Mueller-Hinton broth (MHB, Difco) but MHB supplemented with 5% Fildes enrichment for *Haemophilus influenza*. The overnight culture was diluted 1:1000 to yield a bacterial cell concentration of approximately 1×10^6 CFU/mL.

Preparation of agar plates containing the compounds

Agar plates containing the serial 2-fold dilutions of the compound solutions were prepared for minimal inhibitory concentration (MIC) determination. In this procedure, 9 mL of melted agar medium (Sensitivity Disk Agar, Nissui) with growth supplements were mixed with 1 mL of the compound solution and then solidified at room temperature. For preparation of compound-free plates used as a growth control, 1 mL of DW was added to the medium instead of the compound solution. The final concentration range of the compounds was 128 - 0.004 μg/mL.

Inoculation and incubation

Inoculation was performed by the inoculum replicator and then the plates were incubated at 37°C for 20 hr. The final concentration of inoculum on the agar was approximately 10^3 CFU/spot.

Determining MIC end points

The minimal inhibitory concentration (MIC) was determined as the lowest concentration of the compound that inhibited growth of the organism as detected by the unaided eye.

EXPERIMENTAL RESULTS

Appendix A presents minimum inhibitory concentrations (MIC) for each of the compounds A, B and C in each of the nine series against each of the fifteen tested bacterial strains.

In each of the nine series, MIC were compared between compounds A, B and C for each of the fifteen tested bacterial strains except for 11) *Pseudomonas aeruginosa* SR5393 (P. aeruginosa SR5393) in the ninth series because the data for the compound 9-A were inadvertently omitted. Thus, total 134 = (9×15) – 1 comparisons were performed.

COMPARISON OF BROAD INHIBITORY ACTIVITY

Compound A demonstrated MIC that is lower or the same as MICs of both corresponding compounds B and C, i.e. compounds B and C from the same series, in 125 out of the 134 cases. In other words, compound A had MIC higher than that of either the corresponding compound B or the corresponding compound C only in 10 of the 134 cases. In each of such 10 cases, compound A had MIC 2 times higher than the corresponding compound B or C that demonstrated the lowest MIC within the series. In none of the cases, compound A had MIC more than 2 times higher than that of the corresponding compound B or C that demonstrated the lowest MIC within the series.

Compound B demonstrated MIC that is lower or the same as MICs of both corresponding compounds A and C in 68 out of the 134 cases. In other words, compounds B have MIC higher than MIC of either the corresponding compound A or the corresponding compound D in 67 of the 134 cases. In 20 out of such 67 cases, compound B had MIC 4 or

more times higher than that of the corresponding compound A or C that demonstrated the lowest MIC within the series.

Compound C demonstrated MIC that is lower or the same as MICs of both corresponding compounds A and B in 62 out of the 134 cases. In other words, compounds C had MIC higher than MIC of the corresponding compound A or the corresponding compound B in 73 of the 134 cases. In 18 out of such 73 cases, compound C had MIC 4 or more times higher than that of the corresponding compound A or B that demonstrated the lowest MIC within the series.

COMPOUNDS A VERSUS COMPOUNDS B

Compounds A and B demonstrated comparable potent inhibitory activity against cephem sensitive bacterial strains 5) E. coli NIHJ JC-2, 7) E. cloacae ATCC 13047, and 9) P. aeruginosa SR24.

In particular, for the strain 5), compounds A and B had the same MIC for four out of the nine series. In the remaining five series, compound A had MIC two times higher than the corresponding compound B.

For the strain 7), compounds A and B had the same MIC for six out of the nine series. In the remaining three series, compound A had MIC two times lower than the corresponding compound B.

For the strain 9), compounds A and B had the same MIC in seven out of the nine series. In one of the remaining two series, compound A had MIC two times lower than the corresponding compound B, while in the other of the remaining two series, compound A had MIC two times higher than the corresponding compound B.

At the same time, compounds A demonstrated superior inhibitory properties compared to their respective compounds B against cephem resistant strains 8) E. cloacae RS4321(Bla++), 10) P. aeruginosa SR24-12 (Bla+) and 14) H. influenzae SR11435(BLNAR).

In particular, for the strain 8), compound A had MIC 4 or more times lower than that of the corresponding compound B in eight out of the nine series. In the remaining one series, compound A had MIC 2 times lower than the corresponding compound B.

For each of the strains 10) and 14), compound A had MIC 4 times lower than that of the corresponding compound B in five out of the nine series and 2 times lower in the remaining four series.

COMPOUNDS A VERSUS COMPOUNDS C

Compounds A and C demonstrated comparable potent inhibitory activity against cephem resistant bacterial strains 8) E. cloacae RS4321(Bla++), 10) P. aeruginosa SR24-12 (Bla+) and 14) H. influenzae SR11435(BLNAR).

In particular, for the strain 8), compounds A and C had the same MIC in four out of the nine series, in the remaining five series, compound A had at least 2 times lower MIC than the corresponding compound C.

For the strain 10), compounds A and C had the same MIC in two out of the nine series. In the remaining seven series compound A had two times lower MIC than the corresponding compound C.

For the strain 14), compounds A and C had the same MIC in seven out of the nine series. In one of the remaining two series, compound A had 2 times lower MIC than the corresponding compound C, while in the other of the remaining two series, compound C had 2 times lower MIC than the corresponding compound A.

At the same time, compounds A demonstrated superior inhibitory properties compared to their respective compounds C against cephem sensitive strains 5) E. coli NIHJ JC-2, 7) E. cloacae ATCC 13047, and 9) P. aeruginosa SR24.

In particular, for the strain 5), compounds A had MIC at least 4 times lower than that of the corresponding compounds C in each of the nine series.

For the strain 7) compound A had MIC 4 or more times lower than that of the corresponding compound C in six out of the nine series. In the remaining three series, compound A had MIC 2 times lower than that of the corresponding compound C.

For the strain 9), compound A had MIC 4 times lower than that of the corresponding compound C in five out of the nine series. In the remaining four series, compound A had MIC 2 times lower than that of the corresponding compound C.

CONCLUSION

1) Each of compounds A demonstrated an overall inhibitory activity against a broad spectrum of bacterial strains unexpectedly superior to that of its respective compounds B and C. Differences in the overall inhibitory activity against the broad spectrum of bacterial strains of compounds A on one hand and either of their respective compounds B and C on the other hand are statistically significant.

2) Each of compounds A had the inhibitory activity against cephem resistant bacterial strains 8) E. cloacae RS4321(Bla++), 10) P. aeruginosa SR24-12 (Bla+) and 14) H. influenzae SR11435(BLNAR) unexpectedly superior to that of its respective compound B, while the inhibitory activity against cephem sensitive bacterial strains 5) E. coli NIHJ JC-2, 7) E. cloacae ATCC 13047, and 9) P. aeruginosa SR24 of compounds A and their respective compounds B was similar. Difference between compounds A and their respective compounds B in the inhibitory activity against cephem resistant bacterial strains 8) E. cloacae RS4321(Bla++), 10) P. aeruginosa SR24-12 (Bla+) and 14) H. influenzae SR11435(BLNAR) is statistically significant.

3) Each of compounds A had the inhibitory activity against cephem sensitive bacterial strains 5) E. coli NIHJ JC-2, 7) E. cloacae ATCC 13047, and 9) P. aeruginosa SR24 unexpectedly superior to that of its respective compound C, while the inhibitory activity against cephem resistant bacterial strains 8) E. cloacae RS4321(Bla++), 10) P. aeruginosa SR24-12 (Bla+) and 14) H. influenzae SR11435(BLNAR) of compounds A and their respective compounds C was similar. Difference between compounds A and their respective compounds C in the inhibitory activity against cephem sensitive bacterial strains 5) E. coli NIHJ JC-2, 7) E. cloacae ATCC 13047, and 9) P. aeruginosa SR24 is statistically significant.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

August 21, 2007 — Y. Yamano

Date — YOSHINORI YAMANO